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Augmentative releases of parasitoid wasps in stored wheat reduces insect fragments in flour

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Abstract

Field studies were conducted to assess the effectiveness of the parasitoid wasp, *Theocolax elegans*, for reducing insect fragments in flour by suppressing populations of *Rhyzopertha dominica* in six bins, each containing 27 tonnes of wheat. Beetles were released into all the six bins at monthly intervals for 3 months. Parasitoid wasps were released into three of the bins, 21 days after the first beetle release. Wheat samples from the bins were milled to determine the effects of parasitoid releases on insect fragment counts in flour. In the first year of the study, after 198 days of storage, insect fragment counts were 9.4 and 31 per 50 g flour in the treatment and control bins. However, because of high variability, the means were not significantly different. New grain was used in the second year of the study, and higher numbers of beetles were released. After 131 days of storage, fragment counts averaged 56 and 487/50 g in the treatment and control bins, a reduction in the former of 89%. In the second year of the study, insect myosin in the treatment and control bins averaged 0.27 and 3.23 ng/well, a percentage reduction in the treated bin of 92%. The number of insect damaged kernels (IDK) was significantly lower in the treatment than in the control bins in both years of the study. In the first year, IDK was 6 and 15 IDK/100 g wheat in the treatment and control bins respectively, a reduction in the former of 61%. In the second year, IDK was 12 and 148 IDK/100 g wheat, in the treatment and control bins respectively, a reduction of 92%. This study showed that augmentative releases of parasitoid wasps into bins of stored wheat reduced damage to wheat kernels and the number of insect fragments in flour. Published by Elsevier Science Ltd.

Keywords: *Rhyzopertha*; *Theocolax*; Parasitoid; Biological control; Insect fragments; Stored grain

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1. Introduction

Biological control is an often-underutilized component of integrated pest management of stored grain. Grain managers tend to look only at chemical alternatives to control insects in stored grain. The use of natural enemies to control stored-grain insect pests may seem relatively new but biological control was used as far back as 1911 to control Mediterranean flour moth (Froggatt, 1912). Previous reviews of biological control in stored-products include those by Schöller et al. (1997), Haines (1984) and Brower et al. (1996). Interest in non-chemical methods of controlling insects in stored grain is increasing, as consumers become less tolerant of pesticide residues in their food. Recent legislation in the USA has allowed for augmentative releases of beneficial insects in stored products. All genera of parasitoids and predators known commonly to attack stored-food insect pests were exempted by the Environmental Protection Agency (EPA) from a tolerance requirement in stored raw whole grains and packaged food in warehouses so long as the insects do not become a component of the food (Anonymous, 1992).

Stored-product insects in the US cause loss of millions of dollars in stored wheat (Harein and Davis, 1992). The lesser grain borer, *Rhyzopertha dominica* (F.), is one of the most common and damaging insect pests of stored wheat in the US. Adults feed primarily on the wheat endosperm and cause considerable damage. If more than 31 insect damaged kernels (IDK) are found per 100 g of wheat, the wheat is classified as sample grade according to US government standards (Anonymous, 1997) and represents a significant loss in value. In practice, US grain mills will usually not accept IDK levels greater than 5/100 g. In addition, *R. dominica* larvae develop within the grain kernel and cannot be removed from the grain by normal cleaning procedures. The resulting insect fragments in grain are also a major concern to millers. The US Food and Drug Administration (FDA) has established a defect action level of 75 insect fragments per 50 g of wheat flour (FDA, 1988), but this level is often higher than US mills will tolerate.

Theocolax elegans (Westwood) is a small pteromalid wasp that attacks *R. dominica* and *Sitophilus* spp. (Goodrich, 1921). In large-scale field studies, Flinn et al. (1996) showed that *T. elegans* was very effective in suppressing *R. dominica* populations in 27 tonnes bins of stored wheat. This wasp normally parasitizes larvae that are feeding inside the grain kernel (Goodrich, 1921). Although wasp larvae can complete development on 3rd instar and prepupal *R. dominica*, *T. elegans* larval survivorship is highest when 4th instar *R. dominica* are parasitized. They normally lay one egg externally on each host (Sharifi, 1972). At 32°C, it takes about 15 days to complete development on *R. dominica* (P.W. Flinn, unpublished data). The generation time of this wasp is about half that of *R. dominica*. If hosts are available, female wasps live for 10–20 days at 32°C. A single female *T. elegans* can parasitize up to six *R. dominica* per day (P.W. Flinn, unpublished data). These hymenopterous parasitoids are very small (1–2 mm), and do not feed on the grain. They will normally die within 5–10 days if no beetles are present in the grain. These parasitoids are found naturally in stored grain, which suggests that once released they may continue to suppress pests for many years. Because the adult wasps are external to the grain, they can easily be removed using normal grain-cleaning procedures.

The objective of this study was to determine whether the reduced population of *R. dominica*

in wheat, caused by augmentative releases of the parasitoid wasp *T. elegans*, would reduce insect fragment counts in flour.

2. Materials and methods

This study is part of an earlier study already completed by Flinn et al. (1996); thus, the procedures and materials are only briefly described here. The first experiment was started on 6 July 1993. Six cylindrical steel bins, 4.72 m diameter by 3.35 m tall at the eaves, were constructed so that they were airtight, except for two roof ventilation ports that were covered with 80 mesh screen. Each of the six bins was filled with 27.2 tonnes of hard red winter wheat. The depth of the grain was 2 m in each bin. At this time, the grain temperature was $26.7 \pm 0.1^{\circ}C$ ($\pm$ standard error of the mean) and the average grain moisture was $13.2 \pm 0.1\%$. Two hundred and seventy 1-week-old *R. dominica* adults were released on the grain surface of each of the six bins. The same numbers of beetles were released at monthly intervals up to 6 October to simulate beetle immigration, for a total of four releases. Adult parasitoids were released into three of the six bins 21 days after initial beetle infestation. Based on simulations with a model (Flinn and Hagstrum, 1995), 21 days from storage was found to be the best time to start parasitoid releases. Five hundred and forty *T. elegans* adults (all < 3 days old) were added to three of the six bins on 27 July. Model simulations also predicted that releasing twice the number of parasitoids as hosts would result in good control. Two additional parasitoid releases were made in the same bins at weekly intervals after the first release.

The second experiment was started on 7 July 1994. Six bins were each filled with 27.2 tonnes of grain. At this time, the average grain temperature was $26.6 \pm 0.1^{\circ}C$ and grain moisture was $12.8 \pm 0.1\%$. In the second study, we wanted to determine if the wasps could control higher densities of beetles, so we released twice the number of beetles that were used in the first study. We released 540 *R. dominica* into the bins at monthly intervals between 7 July and 7 October, for a total of four releases. On 28 July 1994, 2160 *T. elegans* were released into each of the three treatment bins. On 4 August 1994, we released another 2160 *T. elegans*, and on 8 September 1994, we released a further 2160 *T. elegans* into each of the three treatment bins.

Grain sampling was conducted at monthly intervals using a pneumatic grain sampler (Probe-A-Vac, Cargill, Minneapolis, MN). Seven 3-kg samples in each of three successive 66.6 cm depths of wheat were taken at three points near the center of the bin and at four points 2/3 the distance between the center and the outer wall. Samples were immediately placed in plastic containers. Grain samples were processed over an inclined sieve (89×43 cm, 1.6 mm aperture). Adult insects were identified and counted (live and dead determined).

We used samples from the final sampling dates in 1993 and 1994 to estimate insect fragments in flour, and insect damaged kernels (IDK). Grain moisture was measured with a grain analysis computer (GAC II, Dickey-John, Auburn, IL). The grain samples were cleaned using a Hart-Carter Dockage Tester (Simon-Carter, Minneapolis, MN), then the wheat was milled using a Brabender Quadrumat Senior experimental mill (C.W. Brabender, Hackensack, NJ). Insect fragment analysis was conducted at the Federal Grain Inspection Service in Kansas City, Missouri using the acid hydrolysis method (AOAC, 1965). We also used a commercial

ELISA method that detects insect muscle protein (myosin) (Biotect, Austin, TX) as an additional measure of insect contamination in the flour samples (Quinn et al., 1992).

Means were compared using Mann–Whitney tests (SYSTAT, 1998). Regression analysis tests (SYSTAT, 1998) was used to analyze relationships between adult *R. dominica* density (at the last sampling date) and grain quality factors.

3. Results and discussion

A more detailed analysis of the population dynamics of the parasitoid wasps and beetles was presented in a previous paper (Flinn et al., 1996). Here we present some of the insect density data again because they are relevant to data collected on insect fragment counts in flour.

In the 1993 study, the three treatment bins had lower densities of adult *R. dominica* than the control bins (Fig. 1). Because of high variance, there were no significant differences ($P > 0.05$, $n = 63$) in treatment bins compared with controls for a specific sampling date. However, there was a significant difference when the data were analyzed using the last three sample dates using ANOVA ($F = 4.7$; $df = 1,372$; $P < 0.05$). On the last three sample dates, the control bins averaged 2.0 adult *R. dominica*/kg and the treatment bins averaged 0.2 *R. dominica*/kg. Thus, adult *R. dominica* populations in the treatment bins were suppressed 90% compared with the controls. Lower densities of adult *R. dominica* populations in the treatment bins should lead to lower number of immature *R. dominica*, because *T. elegans* attacks the larval and pupal stages and not the adults.

In the 1994 study, on the last sampling date, the three treatment bins had significantly lower ($P < 0.01$, $n = 63$) densities of adult *R. dominica* than the control bins (Fig. 2). *R. dominica*

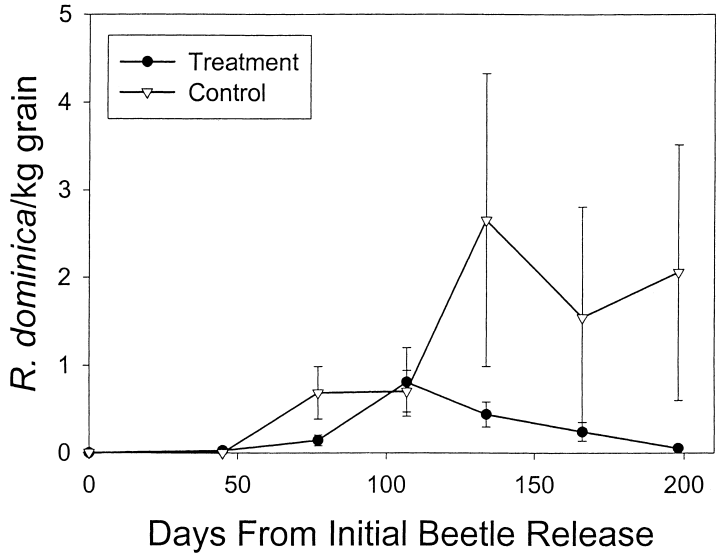

Fig. 1. 1993 average adult *R. dominica* density in three control bins, and in three bins in which the parasitoid *T. elegans* was released. Vertical bars indicate standard error of the mean. Data redrawn from Flinn et al. (1996).

densities in the treatment and control bins, averaged 7/kg and 81/kg, respectively. Thus, adult *R. dominica* populations in the treatment bins were suppressed 91% in comparison with the control bins. The experiment was stopped after 131 days because of the high *R. dominica* density in the control bins.

In general, the number of insect fragments per sample was much lower in the treated than in the control bins in 1993 and 1994 (Fig. 3). *R. dominica* larvae develop inside wheat kernels and were probably the main source of insect fragments in the flour, because the grain was cleaned prior to milling. Grain cleaning would have removed most of the adult *R. dominica* and adult parasitoids. In 1993, fragment counts were 9.4 and 31 per 50 g flour in the treatment and control bins respectively. However, because of high variability, the means were not significantly different ($P > 0.5$, $n = 63$). In the 1994 study, fragment counts were significantly lower ($P < 0.5$, $n = 63$) in the treatment than in the control bins, and averaged 56 and 487/50 g in the treatment and control bins respectively. Thus, insect fragments were reduced by 89% in 1994.

Insect muscle protein (myosin) was also lower in the treatment than in the control bins (Fig. 4). In 1993, the differences between treatment and control were not significantly different ($P > 0.5$, $n = 63$). In 1994, the differences between treatment and control were significantly different ($P < 0.5$, $n = 63$); insect myosin in the treatment and control bins averaged 0.3 and 3.2 ng/well respectively, a percentage reduction of 91% in the treatment bin. Myosin should be a better indicator of insect contamination because it is less variable than insect fragment counts (the number of fragments produced when an insect body breaks up is highly variable, and there is often human error in counting fragments) (Schatzki et al., 1993).

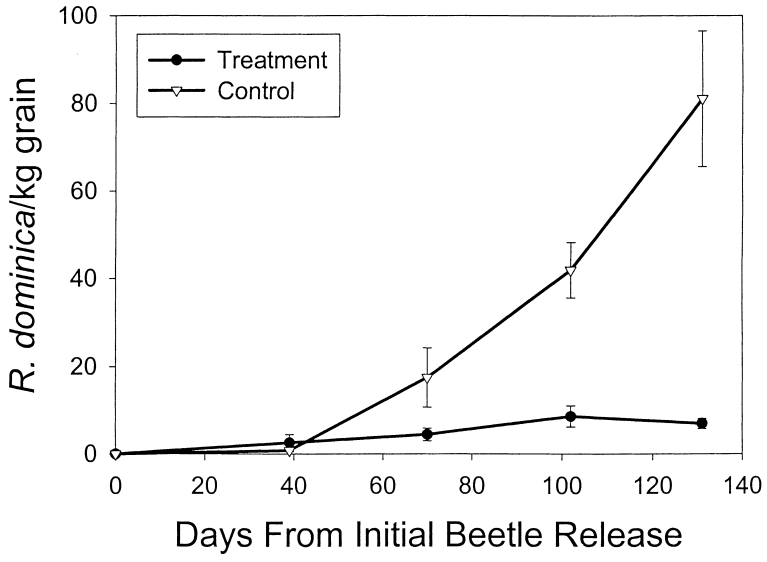

Fig. 2. 1994 average *R. dominica* density in three control bins, and in three bins in which the parasitoid *T. elegans* was released. Vertical bars indicate standard error of the mean. Data redrawn from Flinn et al. (1996).

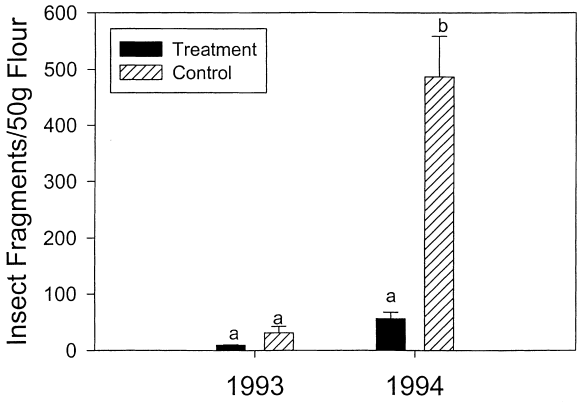

Fig. 3. Mean number of insect fragments in 50 g flour samples milled from wheat obtained from treated and control grain bins. Vertical bars indicate standard error of the mean. Within years, means with different letters are significantly different ($P < 0.05$, $n = 63$).

We used regression analysis to predict insect fragment counts based on myosin concentration. The regression equation was significant ($n = 126$, $P < 0.0001$, $R^2 = 0.59$). Coefficients for the intercept and slope were -5.84 ± 16.44 and 126.33 ± 6.72. This equation can be used to predict insect fragment counts based on the ELISA test for myosin.

The number of IDK was significantly higher ($P < 0.05$, $n = 63$) in the control than in the treatment bins in both years (Fig. 5). An IDK is produced when an internal feeding insect such as *R. dominica* completes its development and exits the kernel, or when an adult *R. dominica* bores into a kernel. In 1993, IDK was about 6 and 15 IDK/100 g wheat in the treatment and control bins, respectively, a percentage reduction in the former of 60%. In the 1994 study, IDK was 12 and 148 IDK/100 g wheat, a percentage reduction of 92%. IDK is an important factor that determines whether grain will be rejected by an elevator or mill. If more than 31

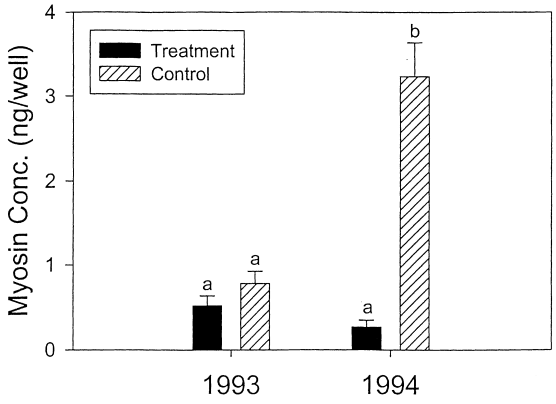

Fig. 4. Mean insect muscle protein (myosin) concentrations (ng/well) from flour samples milled from wheat kernels obtained from treated and control grain bins. Vertical bars indicate standard error of the mean. Within years, means with different letters are significantly different ($P < 0.05$, $n = 63$).

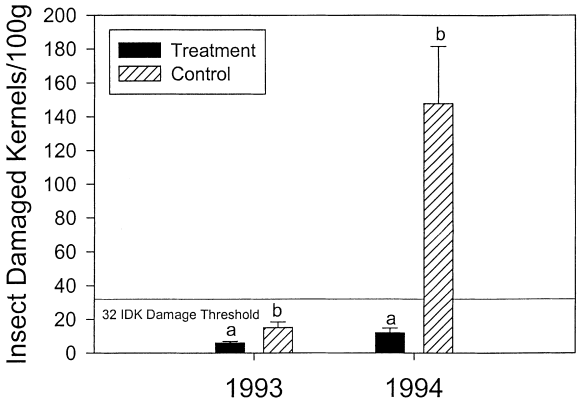

Fig. 5. Mean number of insect damaged kernels per 100 g of wheat obtained from treated and control grain bins. Vertical bars indicate standard error of the mean. Within years, means with different letters are significantly different ($P < 0.05$, $n = 63$).

IDK are found per 100 g of wheat, the wheat is classified as sample grade (according to US government standards).

We used regression analysis to investigate the correlation between live adult *R. dominica* density at the last sampling date and three grain quality factors: fragment count, myosin concentration, and IDK. Treatment and control data were pooled for the analysis. Except for IDK ($P < 0.01$, $R^2 = 0.42$), none of the regressions were significant for 1993. This was probably due to the lower *R. dominica* densities in the 1993 study. For the 1994 study, adult *R. dominica* density was correlated more with myosin concentration $R^2 = 0.65$, than with insect fragments, or IDK (Table 1). Because the grain samples were sieved to remove external insects before milling, myosin and fragment counts are a measure of larval and preemergent adults inside kernels. Thus, samples that had higher external adult *R. dominica* densities also tended to have higher numbers of kernels infested with *R. dominica* larvae or preemergent adults.

This study showed that augmentative parasitoid releases in stored wheat greatly decreased the number of damaged kernels in wheat samples and the number of insect fragments in flour that was milled from this wheat. The insect muscle protein myosin (a better indicator of larvae inside kernels than fragments) was also much lower in milled wheat samples from the treatment bins into which parasitoids had been released.

Table 1
Regression equations for predicting grain quality factors based on live adult *R. dominica* density (adults/kg) in the last sampling date of the 1994 study

	Intercept ± SE[a]	Slope ± SE	P	R^2	n
Fragments	117.25 ± 31.91	3.51 ± 0.31	0.0001	0.51	126
Myosin concentration	0.725 ± 0.160	0.023 ± 0.002	0.0001	0.65	126
IDK	31.73 ± 13.64	1.40 ± 0.16	0.0001	0.48	126

[a] Standard error.

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